Chapter Three

Types and Classification of Bioreactors

Bioreactors can be classified according to various different criteria:

a. Type and form of biocatalyst: free cells in submerged cultures; carried bound or immobilized cells/enzymes; retention or recirculation of the biocatalyst
b. Configuration: tank (height/diameter <3), column (height/diameter > 3)
c. Energy input and aeration: liquid phase; gas phase; combined
d. Hydrodynamics: perfect mixing; partial mixing; no mixing
e. Mode of operation: batch; continuous; fed-batch.

BIOREACTOR DESIGNS

The major types of bioreactors used in industry include:

Stirred tank reactors	In these reactors, mechanical stirrers (using impellers) are used to mix the reactor to distribute heat and materials (such as oxygen and substrates)
Bubble column reactors	These are tall reactors which use air alone to mix the contents

Airlift reactors	These reactors are similar to bubble column reactors, but differ by the fact that they contain a draft tube. The draft tube is typically an inner tube which improves circulation and oxygen transfer and equalizes shear forces in the reactor.
Fluidized bed reactors	In fluidized bed reactors, cells are "immobilized" small particles which move with the fluid. The small particles create a large surface area for cells to stick to and enable a high rate of transfer of oxygen and nutrients to the cells.
Packed bed reactors	In packed bed reactors, cells are immobilized on large particles. These particles do not move with the liquid. Packed bed reactors are simple to construct and operate but can suffer from blockages and from poor oxygen transfer.
Flocculated cell reactors	Flocculated cell reactors retain cells be allow them to flocculate. These reactors are used mainly in wastewater treatment.

Stirred Tank Bioreactors (STB)

Microbial fermentations received prominence during 1940's namely for the production of life saving antibiotics. Stirred tank reactor is the choice for many (more than 70%) though it is not the best. STB's have the following functions:

- Homogenization, suspension of solids, dispersion of gas liquid mixtures, aeration of liquid and heat exchange.
- The STB is provided with a baffle and a rotating stirrer is attached either at the top or at the bottom of the bioreactor.
- Baffles are usually flat vertical plates whose width is about one-tenth of the vessel diameter. Normally, 4-6 baffle plates are fitted to the inside vessel walls to aid mixing and mass transfer by increasing turbulence, preventing vortex formation and eliminating 'dead spaces'.
- Within each vessel the impeller is connected to an external motor, which drives the stirrer system.

- The agitator assembly, including the seal, is often a potential route of contamination. To prevent this problem, the shaft has to pass into the fermenter through a set of aseptic seals. There are specific regulations regarding the numbers and types of seals. For certain fermentations, two or three seals are required to minimize the risk of fermenter contamination, and release of microorganisms and their products into the environment.
- The effectiveness of agitation depends upon the design of the impeller blades, speed of agitation and the depth of liquid. Most STRs have height-diameter aspect ratios of 3:1 or 4:1. STRs must create high turbulence to maintain Transfer rates, but this also generates considerable shear force that is detrimental to certain cells. For instance, many animal and plant cells are shear- sensitive and excessive stirring may result in cell disruption. In these cases STRs may be unsuitable without modification and airlift or supported biofilm reactors may be preferred.
- The typical decision variables are: type, size, location and the number of impellers; sparger size and location.
- These determine the hydrodynamic pattern in the reactor, which in turn influence mixing times, mass and heat transfer coefficients, shear rates etc.
- The conventional fermentation is carried out in a batch mode. Since stirred tank reactors are commonly used for batch processes with slight modifications, these reactors are simple in design and easier to operate.
- Many of the industrial bioprocesses even today are being carried out in batch reactors though significant developments have taken place in the recent years in reactor design, the industry, still prefers stirred tanks because in case of contamination or any other substandard product formation the loss is minimal.
- The batch stirred tanks generally suffer due to their low volumetric productivity. The downtimes are quite large and unsteady state

fermentation imposes stress to the microbial cultures due to nutritional limitations.

- The fed batch mode adopted in the recent years eliminates this limitation. The STBs offer excellent mixing and reasonably good mass transfer rates.
- The cost of operation is lower and the reactors can be used with a variety of microbial species.
- Since stirred tank reactor is commonly used in chemical industry the mixing concepts are well developed. STR with immobilized cells is not favored generally due to attrition problems; however by separating the zone of mixing from the zone of cell culturing one can successfully operate the system.

Airlift Bioreactors (ALB)

- Airlift bioreactors (ALB) are generally classified as pneumatic reactors without any mechanical stirring arrangements for mixing and use the expansion of compressed gas to bring about the mixing
- The turbulence caused by the fluid flow ensures adequate mixing of the liquid.
- The draft tube is provided in the central section of the reactor.
- The introduction of the fluid (air/liquid) causes upward motion and results in circulatory flow in the entire reactor.
- Even large fermenters doesn't require internal cooling coils as a jacket can normally provide sufficient heat transfer, due to the rapid movement of fluid within the vessel.
- The air/liquid velocities will be low and hence the energy consumption is also low.
- ALBs can be used for both free and immobilized cells.
- There are very few reports on ALBs for metabolite production.
- The advantages of Airlift reactors are the elimination of attrition effects generally encountered in mechanical agitated reactors. It

is ideally suited for aerobic cultures since oxygen mass transfer coefficient are quite high in comparison to stirred tank reactors.

Fluidized Bed Bioreactors (FBB)

Fluidized bed bioreactors (FBB) have received increased attention in the recent years due to their advantages over other types of reactors.

- Most of the FBBs developed for biological systems involving cells as biocatalysts are three phase systems (solid, liquid & gas).
- The FBBs are generally operated in co-current up flow with liquid as continuous phase and other more unusual configurations like the inverse three phase fluidized bed or gas solid fluidized bed are not of much importance.
- Usually fluidization is obtained either by external liquid recirculation or by gas fed to the reactor.
- In the case of immobilized enzymes the usual situation is of two-phase systems involving solid and liquid but the use of aerobic biocatalyst necessitate introduction of gas (air) as the third phase.
- A differentiation between the three phase fluidized bed and the airlift bioreactor would be made on the basis that the latter have a physical internal arrangement (draft tube), which provides aerating and non-aerating zones.
- The circulatory motion of the liquid is induced due to the draft tube.
- Basically the particles used in FBBs can be of three different types:
  i. inert core on which the biomass is created by cell attachment
  ii. Porous particles in which the biocatalyst is entrapped.
  iii. Cell aggregates/ flocs (self-immobilization).
- In comparison to conventional mechanically stirred reactors, FBBs provide a much lower attrition of solid particles.
- The biocatalyst concentration can significantly be higher and washout limitations of free cell systems can be overcome.
- In comparison to packed bed reactors FBBs can be operated with

smaller size particles without the drawbacks of clogging, high liquid pressure drop, channeling and bed compaction. The smaller particle size facilitates higher mass transfer rates and better mixing.

- The volumetric productivity attained in FBBs is usually higher than in stirred tank and packed bed bioreactors.

There are several successful examples of using FBBs in bioprocess development.

Packed Bed Bioreactors

- Packed bed or fixed bed bioreactors are commonly used with attached biofilms especially in wastewater engineering.
- The use of packed bed reactors gained importance after the potential of whole cell immobilization technique has been demonstrated.
- The immobilized biocatalyst is packed in the column and fed with nutrients either from top or from bottom.
- One of the disadvantages of packed beds is the changed flow characteristic due to alterations in the bed porosity during operation.
- While working with soft gels like alginates, carragenan etc. the bed compaction which generally occurs during fermentation results in high pressure drop across the bed.
- In many cases the bed compaction was so severe that the gel integrity was severely hampered. In addition channeling may occur due to turbulence in the bed.
- Though packed beds belong to the class of plug flow reactors in which back mixing is absent in many of the packed beds slight amount of back mixing occurs which changes the characteristics of fermentation.
- Packed beds are generally used where substrate inhibition governs the rate of reaction.
- The packed bed reactors are widely used with immobilized cells.
- Several modifications such as tapered beds to reduce the pressure

drop across the length of the reactor, inclined bed, horizontal bed, rotary horizontal reactors have been tried with limited success.

SOLID-SUBSTRATE FERMENTATIONS

Till now we have discussed fermentation process and the types of reactor in context to liquid fermentation *i.e.* where the fermentation substrate is in liquid form and is also known as submerged fermentation. Another alternative in fermentation is solid substrate fermentation which involves the growth of Microorganisms on solid, normally organic, materials in the absence or near absence of free water. The substrates used are often cereal grains, bran, legumes and lignocellulosic materials, such as straw, wood chippings, etc. Traditional processes are largely food fermentations producing along with compost and silage making. In addition, enzymes, organic acids and ethanol are now produced by solid substrate fermentations. Solid-substrate fermentations lack the sophisticated control mechanisms that are usually associated with submerged fermentations. Their use is often hampered by lack of knowledge of the intrinsic kinetics of microbial growth under these operating conditions. Control of the environment within the bioreactors is also difficult to achieve, particularly the simultaneous maintenance of optimal temperature and moisture. However, in some instances, solid-substrate fermentations are the most suitable methods for the production of certain products. For example, most fungi do not form spores in submerged fermentations, but sporulation is often accomplished in solid substrate fermentations. This method is successfully employed in the production of *Coniothyrium minitans* spores for the biocontrol of the fungal plant pathogen; '*Sclerotinia sclerotiorum*.

Solid-substrate fermentations are normally multistep processing involving:

1. Pretreatment of a substrate that often requires mechanical, chemical or biological processing
2. Hydrolysis of primarily polymeric substrates, *e.g.* polysaccharides and proteins
3. Utilization of hydrolysis products.
4. Separation and purification of end products

Table of Advantages and disadvantages of solid substrate fermentation

Advantages	Disadvantages
Potentially provide superior productivity	Slower microbial growth
Low Cost media	Problems with heat build up
Simple Technology	Bacterial contaminations can be problematic
Low capital cost	Difficulties often uncounted on scale-up
Reduced energy requirements	Substrate moisture level which becomes difficult to control
Lost waste output	
No problems with foaming	

Microorganisms

The microorganisms associated with solid-substrate fermentations are those that tolerate relatively low water activity down to A_w values of around 0.7. They may be employed in the form of:

1. Monocultures, as in mushroom production, *e.g. Agaricus bisporus*;
2. Dual cultures, *e.g.* straw bioconversion using *Chaetomium cellulilyticum* and *Candida tropicalis*; and
3. Mixed cultures, as used in composting and the preparation of silage, where the microorganisms may be indigenous or added mixed starter cultures (inoculants).

Physicochemical Parameters

Water

Water is lost during fermentation through evaporation and metabolic activity. This is normally replaced by humidification or periodic additions of water. If moisture levels are too low, the substrate is less accessible, as it does not swell and microbial growth is reduced. However, if the moisture levels are too high there is a reduction in the porosity of the substrate, lowering the oxygen diffusion rates and generally decreasing gaseous exchange. Consequently, the rate of substrate degradation is reduced and there is also an increased risk of microbial contamination.

Temperature

Heat generation can be more problematic than in liquid fermentations and has a major influence on relative humidity within fermentation. The temperature is largely controlled by aeration and/or agitation of the substrate.

Aeration

Most solid-substrate fermentations are aerobic, but the particular requirements for oxygen depend upon the microorganisms used and the specific process. Rates of aeration provided are closely related to the need to dissipate heat, CO_2 and other volatile compounds that may inhibitory. The kinetics of oxygen transfer in solid substrate fermentations is poorly understood. However, the rate of oxygen transfer is greatly influenced by the size of the substrate particles, which determines the void space. Oxygen transfer within this void space is closed related to the moisture level, as the oxygen dissolves in the water film around the substrate particles: However, as mentioned above, if excess water *fills* the void spaces, it has a detrimental effect on oxygen transfer.

Bioreactors Used for Solid-substrate Fermentations

Most solid-substrate fermentations are batch processes although attempts are being made to develop semi-continuous and continuous systems. Some processes do not require bioreactors; they simply involve spreading the substrate onto a suitable floor. These processes employing vessels exhibit considerable variations. A few anaerobic processes, such as silage production, require no mechanisms for agitation or aeration. However, the majority are aerobic fermentations, requiring aeration and occasional or continuous agitation. Bioreactors commonly used include the following:

1. *Rotating drum fermenters*, comprising a cylindrical vessel of around 100 L capacities mounted on its side onto rollers that both support and rotate the vessel. These fermenters are used in enzyme and microbial-biomass production. Their main disadvantage is that the drum is filled to only 30% capacity, otherwise mixing is inefficient.
2. *Tray fermenters*, which are used extensively for the production of fermented oriental foods and enzymes. Their substrates are spread onto each tray to a depth of only a few centimeters and then stacked in a chamber through which humidified air is circulated. These systems

require numerous trays and large volume incubation chambers of up to 150-m^3 capacities

3. *Bed systems*, as used in commercial koji production consisting of a bed of substrate up to 1 m deep, through which humidified air is continuously forced from below
4. *Column bioreactors*, consisting of a glass or plastic column, into which the solid substrate is loosely packed, surrounded by a jacket that provides a means of temperature control. These vessels are used to produce organic acids, ethanol and biomass.
5. *Fluidized bed reactors*, which provide continuous agitation with forced air to prevent adhesion and aggregation of substrate particles. These systems have been particularly useful for biomass production for animal feed.

ANAEROBIC REACTORS

Anaerobic reactors are generally used for the production of methane rich biogas from manure (human and animal) and crop residues. They utilize mixed methanogenic bacterial cultures which are characterized by defined optimal temperature ranges for growth. These mixed cultures allow digesters to be operated over a wide temperature range *i.e.* above 0°C up to 60°C. When functioning well, the bacteria convert about 90% of the feedstock energy content into biogas (containing about 55% methane), which is a readily use able energy source for cooking and lighting. The sludge produced after the manure has passed through the digester is non-toxic and odorless. Also, it has lost relatively little of its nitrogen or other nutrients during the digestion process thus, making a good fertilizer. In fact, compared to cattle manure left to dry in the field the digester sludge has higher nitrogen content; many of the nitrogen compounds in fresh manure become volatized whilst drying in the sun. On the other hand, in the digested sludge little of the nitrogen is volatized, and some of the nitrogen is converted into urea. Urea is more readily accessible by plants than many of the nitrogen compounds found in dung, and thus the fertilizer value of the sludge may actually be higher than that of fresh dung. Reliability problems have arisen from a number of problems *i.e.* construction defects, the mixed nature of the bacterial population, the digesters requirements for water and the maintenance of the optimum nitrogen ratio of the medium. Modern designs have answered

many of these problems and digesters are again becoming useful, especially with regard to the potential of digesters to remove toxic nutrients such as nitrates from water supplies; levels of which are now much more stringently controlled in many industrialized countries. The combination of energy production with the ability to enhance crop yields makes biogas technology a good candidate for more widespread use now that reliable operation can be demonstrated.

SELECTION OF BIOREACTOR

The preliminary choice of bioreactor depends on the number of factors some of which are listed below in the table.

Reactor and processing Area or application (research, production)	reactor dimensions, material, mode of operation, instrumentation
Value or desired product	material, size, mode or operation, instrumentation
Processing (batch, fed- batch, continuous)	reactor dimensions, combination or reactors, instrumentation
Subsequent downstream processing	mode or operation, auxiliary devices, special designs, membrane reactors
Product formation kinetics	mode or operation, reactor type, reactor combination, flow patterns
Culture and culture medium Metabolic state (aerobic, microaerobic, anaerobic)	oxygen input, reactor type, seals
Physiological state (viable, dead, growing, resting)	cell retention, oxygen input
Morphology (spherical, filamentous, flocs, films)	dispersing device, energy input, reactor geometry, reactor type
Sterility (sterile, nonsterile)	materials, fittings, seals, sterile techniques, sampling, auxiliary devices
Properties Physicochemical (gaseous, liquid, solid, multiphase)	dispersing device, internals, cooling, processing
Biological (inhibition, mechanical stress)	substrate dosage, energy dissipation
Rheological (high, low viscosity)	dispersing device, reactor type and geometry, internals
Bubbles (promoting or inhibiting coalescence)	shape and design or gassing device, foam control, bubble dispersion
Foam formation	gas sparger, reactor design, flow patterns, antifoaming agent